INFLUENCES OF SOME ESSENTIAL OILS ON VASE-LIFE OF *GLADIOLUS HYBRIDA*, L. SPIKES.

By

Hegazi, M. A.[1] and El-Kot, G. A. N.[2]

[1]Dept. Hort. Floriculture, [2]Dept. Agric. Botany (Plant Pathology), Fac. Agric., Kafr El-Sheikh University, Egypt

ABSTRACT

Essential oils of clove, cinnamon, ginger, marjoram and fennel were evaluated against microorganisms grown in preservative solution of *Gladiolus hybrida* cv. Peter pears spikes compared with $AgNO_3$ at 10 ppm and distilled water. The essential oils were used as additives to preservative solution at 500 ppm. Data showed that, using essential oils in preservative solution significantly decreased the microbial density, spike base rot and deteriorated florets and increased spikes vase-life. The highest number of open florets and spikes water uptake was recorded when $AgNO_3$ and fennel oil were added to preservative solution as compared with control. Although some essential oils i.e. clove and fennel surpassed $AgNO_3$ in some characters, $AgNO_3$ prolonged the flower longevity in vase more than all essential oils.

Keywords: *Gladiolus hybrida*, essential oils, Biocontrol, vase-life.

INTRODUCTION

Gladioli (*Gladiolus hybrida* cv. Peter pears) is a genus of flowering bulbs in the iris Family (Iridaceae) which sometimes called the sword lily and native to South Africa. The species vary from very small to the spectacular giant flower spikes in commerce. The flowers are variously colored, pink to reddish or light purple with white, contrasting markings, or white to cream or orange to red. Among the most common reasons for early senescence of fresh cut spikes are both the inability of spikes to absorb water due to their rots, blockage and the short supply of carbohydrates to support respiration. Microorganisms specially bacteria which grown in preservative solution and play a great role in vase-life of flowers. The high bacterial counts in water reduced the longevity of the cut flowers (Zagory and Reid, 1986, Doorn and Witte, 1994, Stamps and McColley, 1997 and Loon *et al.* 1998). These microorganisms and their chemical products plug the stem ends and restrict water absorption. They

continue to multiply inside and eventually block the stem tubes and finally decreased longevity of flowers (Doorn *et al.*, 1991 and Doorn *et al.* 1995). The water conducting tubes in the stem (xylem vessels) become plugged by bacteria, yeast, and/or fungi, which are living in the water or on the flower, and proliferate in the containers holding the flowers (Doorn, 1997).

Essential oils have been investigated for their antimicrobial properties against some bacteria and fungi. The main constituents of the used essential are phenolic compounds, the antifungal mechanisms of some essential oils is due to the synthetic inhibition of DNA, RNA, Protein and polysaccarides (Xia *et al.*, 1995 and Gogoi *et al.*, 1997). Bouchra *et al.* (2003) reported that oil constituents such as carvacrol and thymol are strong inhibitors of most pathogenic fungi. Similarly, the volatile terpenes of some essential oils such as carvacrol, ρ-cymene and thymol are considered antifungal activity. Essential oils could be used in preservative solution as well as on fruits for controlling fungal and bacterial pathogens (Daferera *et al.*, 2002, Arouee and Zangoiee, 2006 and Arouiee *et al.*, 2007).

This research aims to study the effect of some essential oils as additives to preservative solution on the microbial density in preservative solution in order to prolong the vase-life of Gladioli spikes.

MATERIALS AND METHODS

This trial was carried out to study the effect of some essential oils i.e., clove, cinnamon, ginger, marjoram and fennel as additive to preservative solution on microbial density, vase-life and quality parameters of gladioli spikes using two batches, the first on January^{2nd} and the second on Jul^{15th} during 2008.

I. Preparation of the used essential oils:

Essential oils were hydro distilled in a Clevenger's type apparatus for 6 hrs at the laboratory of Ornamental and Medicinal branch, Horticulture Department, Faculty of Agriculture, Kafr El-Sheikh University. The extracted oils were dried over anhydrous sodium sulphate to remove traces of moisture then stored in a refrigerator in the dark at 4 °C until use (European Pharmacopoeia procedure, 1983). Tween-20 "0.1% v/v" was used to dissolve the oils before adding to the preservative solution at 500 ppm (Arouiee *et al.*, 2007).

II. Plant material and vase establishment:

Uniform gladioli (*Gladiolus hybrida* cv. Peter pears) spikes free from diseases, mechanical injury and insect injuries were obtained from local wholesale distributor. Spikes were cut under aseptic conditions to 60 cm length. Gladioli spikes were held in glass bottles (3flowers/bottle) containing 250 ml preservative solution including a constant glucose percentage (4%) with 10cm depth. Then the following treatments were conducted:

1. Silver nitrate ($AgNO_3$) as a chemical preservative agent was used at 10 ppm of holding solution.
2. Each of essential oils of clove, cinnamon, ginger, marjoram and fennel was used alone at 500 ppm of preservative solution.
3. Untreated flowers held in preservative solution including a constant glucose percentage (4%) were employed as control treatment. Treatment was presented with three replicates (i.e., 9 cut flowers) spikes were held at room conditions (17±3°C) on January and 28±2°C on July with 60-70% and 70-80% relative humidity respectively. The following parameters were recorded:

1. Survey of microorganisms:

Microbial survey of the flower stem surface was made just period to vase holding. Samples of lower stem parts (10cm) were rinsed in 50 ml sterilized distilled water with shaking for 15 min. screening of microorganisms in holding solution was done at the beginning of wilting using serial dilutions. Portions of the obtained suspensions (0.1 ml) were plated on to standard-plate-count agar medium for bacterial count (Seeley and Vandemark, 1981) and malt agar medium for counts of fungi and yeasts (Abd El-Hafez *et al.,* 1996). Counts were registered as CFU (colony forming unit) after 72 h incubation period at 27±1°C. Samples of the arising single colonies with different phenotypic and microscopic features were picked up on slants for further possible identification studies. Identification of the isolated fungi, yeasts and bacteria were carried out at microbiological branch, Agricultural Botany Department, Faculty of Agriculture, Kafr El-Sheikh University according to Alexopoulus, 1979 for fungi, Barnett and Pankhurst, 1974 for yeasts and Bergy's manual for bacteriology, 1984.

2- Spike base rot caused by microorganisms in preservative solution were recorded as cm rot on Gladioli spikes.

3. Spikes vase life:

The data of spikes were recorded four times with three days intervals. The total numbers of both opened and deteriorated florets were recorded for times after 3, 6, 9 and 12 days from the beginning of experiment and percentage of deteriorated /opened florets were calculated. Vase life of each spike was considered terminated when the number of deteriorated florets surpassed the number of the opened ones.

4. Water uptake and spike fresh weight losses:

Water uptake was used as a criterion for spike quality by determination the relationship between the cumulative water absorption of each individual spike and the time after beginning of the experiment. Also the loss in spike fresh weight with the time was determined.

5. Mean of opened florets number.

6. Mean of deteriorated florets number.

7. Percentage of mean of deteriorated florets number/ mean of opened florets number.

8. Total carbohydrates

At the end of the experiment, spike total carbohydrates were determined according to Herbert *et al.* (1971).

Means between treatments were compared with Duncan's Multiple Range Test according to Snedecor and Cochran (1982).

RESULTS AND DISCUSSION

1. Microbial survey and spike base rot:

Using essential oils of clove, cinnamon, ginger, marjoram and fennel as preservative agents in holding solution have various effects on microbial counts (Table, 1). Generally, the used essential oils significantly reduced microbial density in holding solution compared with distilled water. The high inhibitor of microbial counts appeared when AgNo3 at 10 ppm was added to holding solution followed by clove oil at 5oo ppm in this respect when compared with the other used essential oils and distilled water.

Data presented in Table (1) showed that numerous microorganisms could be isolated from spike surface and preservative solution. In respect of dominance, on the spike surface yeasts came first followed by bacteria and then fungi. The most frequent genera of fungi which isolated from spike surface and preservative solution belonged to *Botrytis, Fusarium, Mucor, Rhizopus, Aspergillus* and *Cladosporium.* While, the most

isolated genera of the yeasts were *Rhodotorula* and *Torulopsiss*. The used essential oils were significantly minimize microbial counts in preservative solution specially when essential oil of clove was added to preservative solution at 5oo ppm. This finding may be due to that essential oils contained specific components or antifungal compounds as phenol agents that can inhibit the growth of certain microorganisms (Farag *et al.*, 1989). Generally, the bacterial percentages exceed those of fungi in the preservative solution of the control treatment. This may suggests that sap exudates of plant materials in preservative solution and/or the vase environment may enhance the bacterial growth rather than the fungal growth (Woltering, 1987). Reviewing the isolated microorganisms, it was observed a qualitative but not quantitative resemble between those isolated from spike surface and those from the preservative solution of the control treatments. This leads to presume that plant materials are the source of vase including contamination (Menesy and El-Gremi, 1996). The obtained results confirmed that the preservative agents extended the vase-life of gladiolus spikes by acting as a microbial inhibitor in the preservative solution (Zagory and Ried, 1986).

Table (1): Effect of essential oils of clove, cinnamon, ginger, marjoram and fennel on microbial counts (CFU/ml) in preservative solution of *Gladiolus hybrida* spikes.

Treatments	Microbial counts				Inhibition (%)	Spike base rot (cm)
	Fungi	yeasts	Bacteria	Total		
Clove oil	43	267	178	488 f	72.56 b	1.1 d
Cinnamon oil	56	306	204	566 d	68.18 d	1.7 b
Ginger oil	62	359	212	633 c	64.41 e	1.8 b
Marjoram oil	49	289	176	514 e	71.10 c	1.3 c
Fennel oil	76	414	232	722 b	59.41 f	1.8 b
$AgNO_3$	2	7	13	22 g	98.76 a	0.5e
Distilled water	166	1271	342	1779 a	00.00 g	3.1 a

The number in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 level.

2. Vase life and water uptake of gladiolus spikes:

Data in Table (2) show that all used treatments have prolonged gladiolus spikes vase life in both batches compared with distilled water. The longest vase life is obtained from holding spikes in preservative solution containing $AgNO_3$, clove oil, marjoram and cinnamon oils,

since they recorded 15.67, 13.67, 12.67 and 12.00 days, respectively in the first batch. Whereas, the best treatments in the second batches were $AgNO_3$, clove and cinnamon oil.

In respect of water uptake, data in Table (2) revealed that, the cumulative water absorption with the time was highest with holding spikes in preservative solution containing $AgNO_3$, clove oil, fennel oil marjoram oil and cinnamon oil which gave 220.33, 220.00, 215.33, 210.33 and 200.67 ml, respectively in the first batch however, the treatment of $AgNO_3$ followed by marjoram oil then clove oil in the second batch gave 231.00, 215.33 and 207.00 ml, respectively. This ability to inhibition of microorganisms resulted in prolong vase-life of gladiolus spikes. $AgNo_3$ prolonged the flower longevity more than used essential oils. This may be due to an alternative role of $AgNo_3$ as inhibitor to ethylene production and action (Nowak and Rudnicki, 1990).

Table (2): The effect of clove, cinnamon, ginger, marjoram and fennel essential oils as additive to preservative solution on vase life and water uptake of *Gladiolus hybrida* spikes.

Treatments	Vase life (days)		Water uptake (ml)	
	1st batch	2nd batch	1st batch	2nd batch
Clove oil	14.33 f	11.00 ef	220.00 j	207.00 g
Cinnamon oil	12.00 e	10.33 de	200.67 g	189.00 d
Ginger oil	9.33 c	10.00 de	158.00 e	202.00 f
Marjoram oil	12.67 e	9.67 d	210.33 h	215.33 h
Fennel oil	8.67 bc	8.00 c	215.33 i	195.00 e
$AgNO_3$	15.67 g	14.67 h	220.33 j	231.00 k
Distilled water	7.00 a	6.33 a	100.67 a	84.00 a

The number in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 level.

3. Number of opened florets:

It is quite clear from Fig (1) that addition of essential oils to the vase preservative solution significantly increased the number of opened florets over distilled water at the four record times in both batches. Also, some treatments were similar to or less than $AgNO_3$ in both batches. The highest number of opened florets noticed on treated spikes with marjoram oil after 3, 6 or 9 days either in the first batch or second batch.

4. Number of deteriorated florets:

Data presented in Fig. (2) Showed that the highest number of

deteriorated florets was recorded in control treatment (distilled water). In contrast clove and cinnamon oils significantly reduced the number of deteriorated florets in both batches.

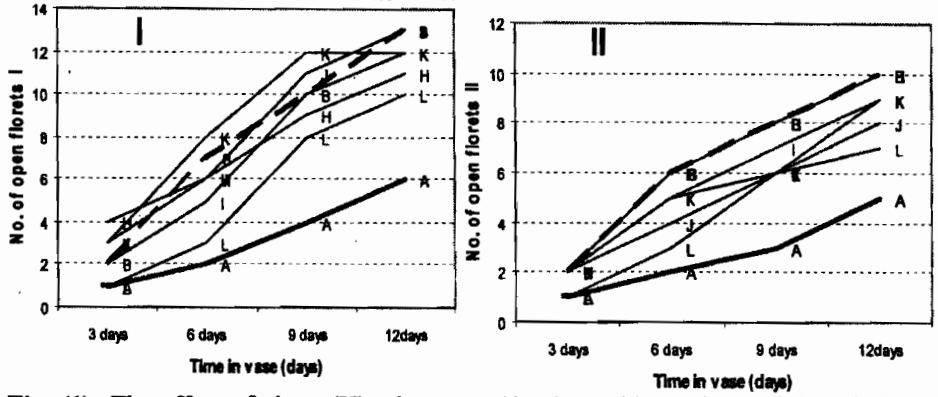

Fig. (1): The effect of clove (H), cinnamon (I), ginger (J), marjoram (K) and fennel (L)essential oils as additive to preservative solution as compared with $AgNO_3$ (B) and distilled water (A) on opened florets of gladiolus spikes during two batches I and II.

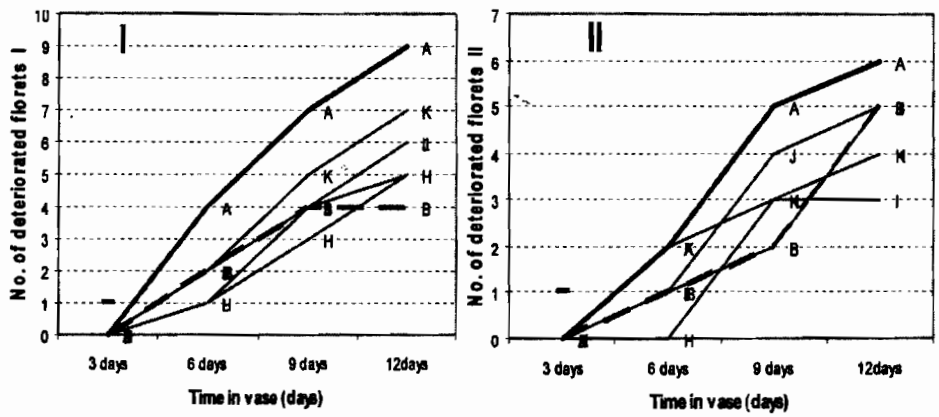

Fig. (2): The effect of clove (H), cinnamon (I), ginger (J), marjoram (K) and fennel (L) essential oils as additive to preservative solution as compared with $AgNO_3$ (B) and distilled water (A) on deteriorated florets of gladiolus spikes during two batches I and II.

5. Deteriorated/ opened florets percentage:

A significant reduction occurred in deteriorated/ opened florets percentage when either used essential oils treatments or $AgNO_3$ were added to the vase solution of gladiolus spikes in both batches (Fig. 3). The highest deteriorated/ opened florets percentage resulted from treated

spikes with distilled water at the four records. Whereas, the lowest percentage were obtained from spikes treated with clove oil and cinnamon oil at 6 days.

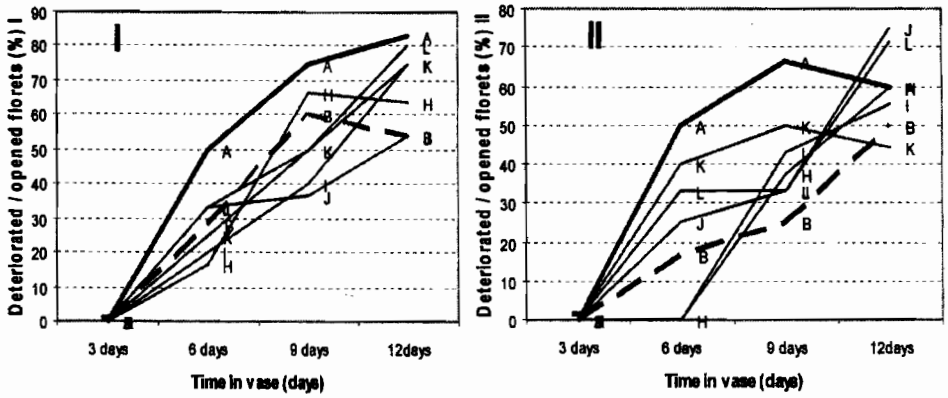

Fig (3): The effect of clove(H), cinnamon(I), ginger(J), marjoram(K) and fennel (L)essential oils as additive to preservative solution compared with $AgNO_3$ (B) and distilled water (A) on deteriorated/ opened flowers percentage during two batches I and II.

Table (3): The effect of clove, cinnamon, ginger, marjoram and fennel essential oils as additive to preservative solution on spike fresh losses **(%)** and total carbohydrates (mg/g D.W.) of *Gladiolus hybrida* spikes.

Treatments	Spike F.W. losses (%)		Total carbohydrates (Mg/g D.W.)	
	1st batch	2nd batch	1st batch	2nd batch
Clove oil	33.11 d	34.52 a	4.03 i	3.44 bc
Cinnamon oil	31.08 c	42.75 f	3.16 e	3.27 bc
Ginger oil	37.10 f	41.92 e	3.90 h	3.15 bc
Marjoram oil	40.55 g	36.87 b	3.11 e	2.88 ab
Fennel oil	35.40 e	47.61 g	3.54 f	2.90 ab
AgNO3	56.00 j	50.77 h	3.87 gh	3.30 bc
Distilled water	72.64 l	78.80 k	2.57 a	2.25 a

The number in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 level.

6. Spike fresh weight losses percentage and Total carbohydrates contents:

Data in Table (3) clear that the highest fresh weight losses were recorded when distilled water was used as a preservative solution for

gladiolus spikes in both batches. There were significant differences among all used essential oils and $AgNO_3$ in most cases. The fresh weight of spikes holed in preservative solution including essential oils continued to increase then declined thereafter due to flower wilting. These results may be explained by the fact that these agents may be increased water absorption which allowed more increase in fresh weight on the contrast of this, distilled water increased bacterial population in the spike subsequently, and it leads to vascular occlusion (Zagory and Reid, 1986). The vascular occlusion causes water stress and spike wilting which results in shorter vase life (Loon *et al.*, 1998).

It is evident from data in Table (3) that total carbohydrates contents in gladiolus spikes treated with distilled water as preservative solution were reduced compared with the other treatments in both batchs. The highest carbohydrates contents in the first batch noticed with spikes treated with clove oil followed by ginger oil and $AgNO_3$ as recorded 4.03, 3.90 and 3.87 mg/g D.W., respectively. Whereas clove oil and $AgNO_3$ were the best treatments at the second one. They recorded 3.44 and 3.30 mg/g D.W., respectively.

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الملخص العربي

تأثيرات بعض الزيوت العطرية على بقاء شماريخ الجلاديولس في الفازة

محمود عبدالنبى حجازى* ، جبر عبد الوئيس نصر القط**
كلية الزراعة- جامعة كفر الشيخ
*= قسم البساتين (شعبة الزينة) **= قسم النبات الزراعي (شعبة أمراض النبات)

تم تقييم استخدام الزيوت العطرية لكل من القرنفل، القرفة ، الزنجبيل، البردقوش والشمر في مقاومة الميكروبات النامية في محلول الحفظ لشماريخ الجلاديولس مقارنة باستخدام نترات الفضة بتركيز ١٠ جزء في المليون. استخدمت الزيوت كإضافات لمحلول الحفظ بتركيز ٥٠٠ جزء في المليون. حيث أوضحت النتائج أن إضافة الزيوت إلى محلول الحفظ أدى إلى حدوث خفض معنوي في كل من الكثافة الميكروبية، عفن قاعدة الشمراخ وتدهور الزهيرات في حين أدت إلى زيادة مدة بقاء الشماريخ في الفازة. أعطت كل من المعاملة بنترات الفضة و زيت الشمر اعلي عدد للزهيرات المتفتحة وأعلى معدل امتصاص للماء مقارنة بالكنترول.

بالرغم من تفوق بعض الزيوت مثل القرنفل والشمر على نترات الفضة في بعض الصفات، إلا أن نترات الفضة أطالت بقاء الشماريخ في الفازة أكثر من كل الزيوت.